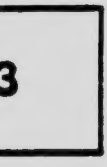

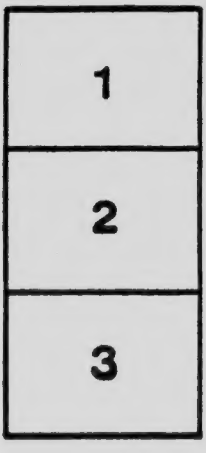

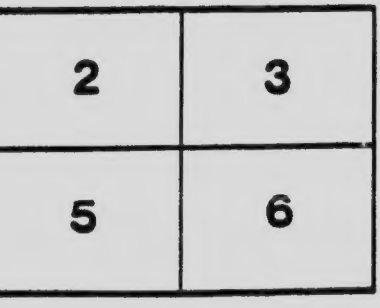

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REPORT No. 6

AN INVESTIGATION INTO THE QUESTION OF EARLY PUTREFACTION OF EVISCERATED FISH IN WHICH THE GILLS HAVE BEEN LEFT

BY

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AN INVESTIGATION INTO THE QUESTION OF EARLY PUTREFACTION OF EVISCERATED FISH IN WHICH THE GILLS HAVE BEEN LEFT.

It is claimed by many fish dealers that eviscerated fish in which the gills have not been taken out putrefy more rapidly than those in which the gills are removed.

In order to determine whether there is a real foundation for this belief, several specimens of pollock and hake were eviscerated; in some the gills were allowed to remain, in others they were removed.

These fish were then exposed to the air in a fairly warm room.

The removed gills were also exposed to the air.

In forty-eight hours a strongly putrefactive odour came from the fish. This appeared to be somewhat more marked from the fish in which the gills were left.

The removed gills which were exposed to the air had dried and showed no evidence of putrefaction.

It seemed, therefore, that the moist gills left in the fish were the seat of fairly active putrefaction.

It remained now to determine the reason for this active putrefaction of eviscerated fish in which the gills are left.

An investigation into the method of eviscerating fish by the fishermen showed that the viscera are carelessly removed by hand, the intestinal contents are smeared over the gills and the fish left for hours without proper cleaning before the dealers receive them.

The question whether the native flora of the gills is more putrefactive than that commonly found on the rest of the fish became unimportant, because the methods of the fishermen quickly insured a rich contamination of every part of the fish with diverse flora.

The problem seemed then to resolve itself into a question of culture medium.

Since every part of the fish was abundantly innoculated with similar bacteria, was the earlier and more extensive putrefaction of the gills due to the fact that gills form unusually good culture

medium for the bacteria? Was it due to the fact that the bloodiness of the gills was conducive to more rapid growth?

To determine this, three sets of media were made:*

(*a*) Fish meat medium (agar and broth).
(*b*) Gill medium (agar and broth).
(*c*) Blood medium (agar and broth).

The following was the method of preparation:

500 gms. of minced fish meat was placed in a pot.

500 cc. of distilled water into which was dissolved 26·5 gms. of sodium chloride, 0·75 gms. of potassium chloride and 3·25 gms. of magnesium chloride, were added to the minced fish meat. The whole was placed in a water bath and gently heated to 40°C for about 20 minutes. The temperature was now suddenly raised to boiling and kept thus for 10 minutes.

This mixture was next strained through butter muslin. Five grams of peptone were now stirred into the fish water and the whole heated at 100°C. for twenty minutes.

The mixture was again filtered and made up to the original 500 cc.

250 cc. of this medium was diluted with an equal volume of distilled water and tubed as fish meat broth.

To the other 250 cc., 4 gms. of agar were added and the mass tubed as fish meat agar for plating.

Another set of media was made in the same way with the exception that minced fish gills were substituted for fish meat. This constituted gill broth and gill agar.

Finally, another set of media in which fish blood was used instead of fish meat constituted blood broth and blood agar.

Thus three sets of media were prepared in exactly the same way and different from one another only in the fact that in the first fish meat was used; in the second fish gills (together with their blood); in the third set fish blood. (Haddock was used in the preparation of these media).

As the same quantities of ingredients were used in each set it was thought reasonable to suppose that the relative cultural values of the various media would resemble those of these tissues in their native state.

* The method is described in Eyre's Bacteriological Technique. W.B. Saunders Co. 1916 p. 190.

Four strains of bacteria in pure culture from different parts of fish were obtained from Miss Eleanor Shanly. These we shall call for convenience sake α, β, γ, and δ *

In order to compare the relative cultural values of the media each set was plated with the four strains of bacteria. In each case the dilutions were made on the broth of the set. Thus α was diluted in fish meat broth and plated in fish meat agar. Similarly with β, γ and δ.*

Next, bacterium α was diluted in gill broth and plated in gill agar. This was also done with β, γ and δ.*

It was then repeated with blood, broth and agar.

The object of plating was to compare the rate of growth of colonies originating from single bacteria in the different media.

Since the technique in plating was carried out with the greatest care, since four different bacteria were used, and as the whole experiment was done in duplicate, it was felt that the rate of appearance of colonies on the plate as well as the size and number of these colonies could be safely interpreted as showing the relative values of the media for cultural purposes.

The observations on the first series of plates after seventy-two hours of cultivation at room temperature are recorded in table A.

TABLE A.

Bacterium.	Fish Meat.	Gill.	Blood.
Alpha.	Numerous colonies. Medium size.	Numerous colonies. Medium size.	Numerous colonies. Medium size.
Beta.	Very few colonies. Small size.	Large number of colonies. Medium size.	Large number of colonies. Medium size.
Gamma.	No visible growth	Very numerous colonies. Medium size.	Large number of colonies. Medium size. Diffuse.
Delta.	No visible growth.	No visible growth.	Fair number of colonies. Medium size.

* See Miss Shanly's report on the intestinal flora of the Sardine Herring, for 1919.

α corresponds to Miss Shanly's 1 gill.
β corresponds to " P I. Intestine.
γ corresponds to " P I. Liver.
δ corresponds to " P I. Stomach.

TABLE B.

Table B represents the results of a duplicate series of plates, i.e. using the same bacteria and the same media.

Bacterium.	Fish Meat.	Gill.	Blood.
Alpha.	Numerous colonies. Medium size.	Numerous colonies. Medium size.	Numerous colonies. Medium size.
Beta.	No visible growth.	Large number of colonies. Medium size.	Large number of colonies. Medium size.
Gamma.	No visible growth.	Very numerous colonies. Good size.	Numerous colonies. Medium and small size.
Delta.	Few colonies. Medium size.	Very few colonies. Medium size.	Fair number of colonies Medium size.

From the above it will be seen that bacterium α is the only one that grows with equal facility on the three media. Bacteria β and γ shows a distinct preference for gill and blood media.

γ shows a particularly good growth on gill medium.

δ appears to grow best on blood medium.

After four days tiny colonies appeared on all the fish meat plates showing that these were not sterile, but that the rate of growth was slower on this medium than on the others.

Thus it is seen that of four bacteria, common found in fish, three show a distinct preference for gills and blood as culture media, and as gills are usually covered with blood and bacteria after the incomplete evisceration that is now in common practice among the fishermen, it is probable that these, i.e. gills and blood, become the seat of an early luxuriant growth of putrefactive organisms.

Guided by these observations it is desirable to recommend the removal of the gills and a thorough washing of the eviscerated fish in order to prevent, at least to some degree, early putrefaction.